

Appl. No. : 10/735,418
Filed : December 11, 2003

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph 10 as follows:

--Further studies of TR3 have yielded a better understanding of its mechanism of action in apoptosis (Li *et al.*, 2000, *Science* 289:1159). First, several apoptosis inducing agents which also induced TR3 expression in human prostate cancer cells were identified. These included the AHPN analog 6-[3-(1-adamantyl)-4-hydroxyphenyl]-3-chloro-2-naphthalenecarboxylic acid (MM11453), the retinoid (Z)-4-[2-bromo-3-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)propenoyl]benzoic acid (MM11384), the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA), the calcium ionophore A23187, and the etoposide VP-16. Second, it was found that the transactivation activity of TR3 is not required for its role in inducing apoptosis, as demonstrated by an experiment that showed that apoptosis inducing agents blocked the expression of a TR3 target reporter gene. This was further supported by the finding that a TR3 mutant deprived of its DNA binding domain (DBD) was still competent for inducing apoptosis. Third, TR3 was found to relocalize to the outer surface of the mitochondria in response to some apoptotic stimuli. TR3, visualized *in vivo* by tagging with Green Fluorescent Protein (GFP), was shown to relocalize from the nucleus to the mitochondria in response to apoptosis-inducing agents. Fractionation studies showed that TR3 was associating with the mitochondria-enriched heavy membrane fraction, and proteolysis accessibility studies on purified mitochondria confirmed that TR3 was associating with the outer surface of the mitochondria, where Bcl-2-family members are also found. Fourth, TR3 was shown to be involved in the regulation of cytochrome c release from the mitochondria. Inhibition of TR3 activity by expression of TR3 antisense RNA blocked the release of cytochrome c and mitochondrial membrane depolarization in cells stimulated with TPA and MM11453. Furthermore, incubating purified mitochondria with recombinant TR3 protein resulted in cytochrome c release.--

Please amend paragraph 36 as follows:

--In addition to peptides consisting only of naturally-occurring amino acids, peptidomimetics or peptide analogs are also considered[[]]. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (see, *e.g.*, Luthman *et al.*, 1996, *A Textbook of Drug Design and*

Appl. No. : 10/735,418
Filed : December 11, 2003

Development, 14:386-406, 2nd Ed., Harwood Academic Publishers; Grante, 1994, *Angew Chem Int Ed Engl*, 33:1699-1720; Fauchere, 1986, *Adv Drug Res*, 15:29; Evans *et al.*, 1987, *J Med Chem* 30:229, all of which are incorporated by reference). Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as naturally-occurring receptor-binding polypeptide, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH₂ NH--, --CH₂ S--, --CH₂ --CH₂--, --CH=CH--(*cis* and *trans*), --COCH₂--, --CH(OH)CH₂--, and --CH₂ SO--, by methods known in the art and further described in the following references: Spatola, 1983, In, *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, B. Weinstein, eds., Marcel Dekker, New York, p. 267; Hudson *et al.*, 1979, *Int J Pept Prot Res* 14:177-185 (1979) (--CH₂ NH--, CH₂ CH₂--); Spatola *et al.*, 1986, *Life Sci* 38:1243-1249 (--CH₂--S); Hann, 1982, *J Chem Soc Perkins Trans I*, 307-314 (--CH--CH--, *cis* and *trans*); Almquist *et al.*, 1980, *J Med Chem* 23:1392-1398 (--COCH₂--); Jennings-White *et al.*, 1982, *Tetrahedron Lett* 23:2533 (--COCH₂--); Szelke, *et al.*, European Appln. EP 45665 (1982) (--CH(OH)CH₂--); Holladay *et al.*, 1983, *Tetrahedron Lett* 24:4401-4404 (--C(OH)CH₂--); and Hruby, 1982, *Life Sci*, 31:189-199 (--CH₂--S--); each of which is incorporated herein by reference. A particularly preferred non-peptide linkage is --CH₂ NH--. Such peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, *etc.*), altered specificity (*e.g.*, a broad-spectrum of biological activities), reduced antigenicity, and others. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (*e.g.*, an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the macromolecules(s) (*e.g.*, immunoglobulin superfamily molecules) to which the peptidomimetic binds to produce the therapeutic effect. Derivitization (*e.g.*, labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic. Generally, peptidomimetics of receptor-binding peptides bind to the receptor

Appl. No. : **10/735,418**
Filed : **December 11, 2003**

with high affinity and possess detectable biological activity (*i.e.*, are agonistic or antagonistic to one or more receptor-mediated phenotypic changes).

Please amend paragraph 56 as follows:

--The peptides typically are synthesized as the free acid but, as noted above, could be readily prepared as the amide or ester. One can also modify the amino and/or carboxy terminus of the peptide compounds to produce other compounds[[]]. Amino terminus modifications include methylation (*i.e.*, --NHCH₃ or --NH(CH₃)₂), acetylation, adding a benzyloxycarbonyl group, or blocking the amino terminus with any blocking group containing a carboxylate functionality defined by RCOO--, where R is selected from the group consisting of naphthyl, acridinyl, steroidyl, and similar groups. Carboxy terminus modifications include replacing the free acid with a carboxamide group or forming a cyclic lactam at the carboxy terminus to introduce structural constraints.

Please amend paragraph 124 as follows:

--To further determine the interaction of DC1 with Bcl-2, a region of hydrophobic amino acids (HRLGCARGFGDWIDSILA) was deleted from TR3/ΔDBD, and the resulting mutant (TR3/ΔDBD/Δ471-488) was analyzed for its interaction with Bcl-2. TR3/ΔDBD/Δ481-488, with eighteen amino acids from 471 to 488 in the DC1 region deleted from TR3/ΔDBD, TR3/ΔDBD/I483A (with Ile483 replaced with Ala) and TR3/ΔDBD/L487A (with Leu487 replaced with Ala) were analyzed for their interaction with Bcl-2 by *in vivo* Co-IP assay as described above. As compared to TR3/ΔDBD, the mutant showed only a very weak weak interaction with Bcl-2. Moreover, a single hydrophobic amino acid mutation (Ile 483 or Leu 487) largely abolished the interaction of TR3/ΔDBD and Bcl-2. These data further confirmed the role of DC1 in mediating the interaction between TR3 and Bcl-2.--